

SEQUENCE LISTING

<110> Kimoto, Norihiro
Yamamoto, Hiroaki
Mitsuhashi, Kazuya

<120> NOVEL CARBONYL REDUCTASE, METHOD FOR PRODUCING SAID ENZYME, DNA
ENCODING SAID ENZYME, AND METHOD FOR PRODUCING ALCOHOL USING SAID
ENZYME

<130> 06501-050001

<140> US 09/468,738

<141> 1999-12-21

<150> JP 1999-171160

<151> 1999-06-17

<150> JP 1998-363130

<151> 1998-12-21

<160> 29

<170> PatentIn Ver. 2.0, reformatted using WordPerfect 5.1

<210> 1

<211> 879

<212> DNA

<213> Kluyveromyces aestuarii

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ttgacagtta tcaactggtg agcaggagcc attggcggag ctctgtgtga gggatttgcg	180
tcctgtggat ctgacgttgt cattttagat taaaaataca gtccctgaatt gtcattcagtt	240
ttggaatcta ggtatggagt gaggtcgaaa agctatcagg tcgacattac gagttcagaa	300
gacgtgaaac ttgttgttgc aaagatttta gaagattttc ctgatcgcca tatcaataca	360
tttgttgcta atgcagggtat tgcattggacc aacgggtcca ttttgaacga aaacgcgacg	420
ccagatgtgt ggaaacgtgt tatggatgtg aacgtgcaag gaacttatca ttgtgcgaaa	480
tatgtggcag aagtgttcaa acaacagggc catggtaatc tgattttgac tgcgtcgatg	540
tcaagttata taagcaacgt tcccaactac caaacatgtt ataattgctc taaagcggcc	600
gtcagacata tggcaaaggg atttgctgtt gaattcgccc atttgacaaa ccccgaggt	660
aaaatcagat gcaattcggg ttcacctggg tacactgaca ccgcactttc agcttttgtt	720
ccggtcgaa acgcgcgtca gtgggtgggga ttgactccta tgggtcgca agcattacca	780
caagagctag tcggagccta ctgttatteg gcattctgac ctgcatcatt cacaaatgga	840
tgtgatattc aagtagacgg tgggtacact tgcgtttga	879

<210> 2

<211> 292

<212> PRT

<213> Kluyveromyces aestuarii

<400> 2

Met Thr Phe Gln His Phe Leu Arg Gly Gly Leu Glu Asp Lys Thr Val
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Pro Gln Glu Pro Pro Lys Glu Gln Tyr Pro Asp Gly Val Asn Tyr Leu

2

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 35 40 45
 Gly Ala Ile Gly Gly Ala Leu Cys Glu Gly Phe Ala Ser Cys Gly Ser
 50 55 60
 Asp Val Val Ile Leu Asp Tyr Lys Tyr Ser Pro Glu Leu Ser Ser Val
 65 70 75 80
 Leu Glu Ser Arg Tyr Gly Val Arg Ser Lys Ser Tyr Gln Val Asp Ile
 85 90 95
 Thr Ser Ser Glu Asp Val Lys Leu Val Val Ala Lys Ile Leu Glu Asp
 100 105 110
 Phe Pro Asp Arg Asp Ile Asn Thr Phe Val Ala Asn Ala Gly Ile Ala
 115 120 125
 Trp Thr Asn Gly Ser Ile Leu Asn Glu Asn Ala Thr Pro Asp Val Trp
 130 135 140
 Lys Arg Val Met Asp Val Asn Val Gln Gly Thr Tyr His Cys Ala Lys
 145 150 155 160
 Tyr Val Ala Glu Val Phe Lys Gln Gln Gly His Gly Asn Leu Ile Leu
 165 170 175
 Thr Ala Ser Met Ser Ser Tyr Ile Ser Asn Val Pro Asn Tyr Gln Thr
 180 185 190
 Cys Tyr Asn Ala Ser Lys Ala Ala Val Arg His Met Ala Lys Gly Phe
 195 200 205
 Ala Val Glu Phe Ala His Leu Thr Asn Pro Ala Gly Lys Ile Arg Cys
 210 215 220
 Asn Ser Val Ser Pro Gly Tyr Thr Asp Thr Ala Leu Ser Ala Phe Val
 225 230 235 240
 Pro Val Glu Gln Arg Ala Gln Trp Trp Gly Leu Thr Pro Met Gly Arg
 245 250 255
 Glu Ala Leu Pro Gln Glu Leu Val Gly Ala Tyr Leu Tyr Leu Ala Ser
 260 265 270
 Asp Ala Ala Ser Phe Thr Asn Gly Cys Asp Ile Gln Val Asp Gly Gly
 275 280 285
 Tyr Thr Cys Val
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<210> 3

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<212> PRT

<213> Kluyveromyces aestuarii

<400> 3

Thr Phe Gln His Phe Leu Arg Gly Gly Leu
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<210> 4

<211> 10

<212> PRT

<213> Kluyveromyces aestuarii

<400> 4

Tyr Ser Pro Glu Leu Ser Ser Val Leu Glu
 1 5 10

<210> 5

<211> 10

<212> PRT

<213> Kluyveromyces aestuarii

<400> 5

Gly Phe Ala Val Glu Phe Ala His Leu Thr
1 5 10

<210> 6

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 6

gacggatcca cwttycarca yttyytragr ggwgg 35

<210> 7

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 7

gtgaagcttc cwccyctyar raartgytgr aawgt 35

<210> 8

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 8

gacggatcct aytstccwga rttrtsttst gtwtrga 38

<210> 9

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 9

gtgaagcttt cyaawacasa asayaaytcw ggasarta 38

<210> 10

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<221> misc_feature

<222> (0)...(0)

<223> n = A, T, G, or C

<400> 10

gacggatccg gwttygcwgt wgarttygcn ca

32

<210> 11

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<221> misc_feature

<222> (0)...(0)

<223> n = A, T, G, or C

<400> 11

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32

<210> 12

<211> 254

<212> DNA

<213> *Kluyveromyces aestuarii*

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aaattgacag ttatcactgg tggagcagga gccattggcg gagctctgtg tgagggattt	180
gcgtcctgtg gatctgacgt tgtcatttta gattacaaat actctcctga attatcttct	240
gtattagaaa gctt	254

<210> 13

<211> 650

<212> DNA

<213> *Kluyveromyces aestuarii*

<400> 13

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aaattgacag ttatcactgg tggagcagga gccattggcg gagctctgtg tgagggattt	180
gcgtcctgtg gatctgacgt tgtcatttta gattacaaat acagtcctga attgtcatca	240
gttttggaat ctaggtatgg agtgagggtcg aaaagctatc aggtcgacat tacgagttca	300
gaagacgtga aacttggtgt tgcaaagatt ttagaagatt ttcctgacg cgatatcaat	360
acatttggtt ctaatgcagg tattgcatgg accaacggtt ccattttgaa cgaaaacgcg	420
acgccagatg tgtggaaacg tgttatggat gtgaacgtgc aaggaaetta tcattgtgctg	480
aaatatgtgg cagaagtgtt caaacaacag ggccatggta atctgatttt gactgcgtcg	540
atgtcaagtt atataagcaa cgttcccaac taccaaacat gttataatgc ctctaaagcg	600
gccgtcagac atatggcaaa gggttttgca gtagagttcg cacaaagctt	650

<210> 14

<211> 437

<212> DNA

<213> *Kluyveromyces aestuarii*

<400> 14
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 gaagattttc ctgacgcga tatcaataca ttgttgtcta atgcaggat tgcattggacc 180
 aacggttcca ttttgaacga aaacgcgacg ccagatgtgt ggaaacgtgt tatggatgtg 240
 aacgtgcaag gaacttatca ttgtgcgaaa tatgtggcag aagtgttcaa acaacagggc 300
 catggtaatc tgattttgac tgcgtcgatg tcaagttata taagcaacgt tcccaactac 360
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 gaattcgctc aaagctt 437

<210> 15
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 15
 tcggtggctc ctgaggaac 19

<210> 16
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Artificially Synthesized Primer Sequence

<400> 16
 acatgttata atgcctctaa agc 23

<210> 17
 <211> 1787
 <212> DNA
 <213> Kluyveromyces aestuarii

<221> misc_feature
 <222> (0)...(0)
 <223> n = A, T, G, or C

<400> 17
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 maagacaagg cgraatagat gacgracgtt ggctgtaaat gtcggggggac naaatagatg 180
 caaatawtws wgmnamywww gkmmymkwyn ttttttaa atgcctggta actacggcag 240
 catgggctcg gtggtagggg aagaacaatt agtctatatt taggagagag gtataaataa 300
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 cgggcctcgt tcacgcgaga gctagctttg cacctgagtt tgggtttaga cacactataa 480
 gaagagttta aagtctagga agtattcaaa aaataaagta aaagtcgcaa tgacgtttca 540
 gcatttttta agaggtggat tagaagataa aacagttcct caggagccac cgaaggagca 600
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gaaacgtgtt atggatgtga acgtgcaagg	aacttatcat tgtgcgaaat atgtggcaga	1020
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aagcaacgtt cccaactacc aaacatgtta	taatgcctct aaagcggccg tcagacatat	1140
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caattcggtt tcacctgggt acactgacac	cgcactttca gcttttgttc cggtcgaaca	1260
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cggagcctac ttgtatttgg catctgacgc	tgcatcattc acaaattggat gtgatattca	1380
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ccccanagtt gcyymymyc ycaaraatga	cmttggttaaw mmsywtgtwr aaacacggca	1560
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aaaagggtgt taatcaaata gacacgcttt	cgaataccatg catcaaacgg tcctaaacag	1680
aacaatacag gacactgtgc agcatggtat	cggcaatcca aagcttcata tccacaagac	1740
tcatacaacga agtcactccc agtcacattt	tttggattta tgcgatt	1787

<210> 18

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 18

tcaggatcca acaatgactt ttcagcattt ttttaag

36

<210> 19

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 19

tgttctagat taaacgcaag tgtaccacc g

31

<210> 20

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 20

tctgtatcag gctgaaaatc ttc

23

<210> 21

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 21
atatattaar. gtrtcgatta aataaggag

29

<210> 22
<211> 891
<212> DNA
<213> Kluyveromyces aestuarii

<400> 22
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cctcaggagc caccgaagga gcaatatccc gatgggtgta attacttgag cttgttcagt 120
cagaaaagga aattgacagt tatcactggt ggagcaggag ccattggcgg agctctgtgt 180
gagggatttg cgtcctgtgg atctgacgtt gtcatttttag attacaaata cagtcctgaa 240
ttgtcatcag ttttggaatc taggtatgga gtgagggtcga aaagctatca ggtcgacatt 300
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actgctcga tgtcaagtta tataagcaac gttcccaact accaaacatg ttataatgcc 600
tctaaagcgg ccgtcagaca tatggcaaag ggatttgcgt ttgaattcgc ccatttgaca 660
aaccgccgag gtaaaatcag atgcaattcg gtttcacctg gttacactga caccgcactt 720
tcagcttttg ttccggtcga acagcgcgt cagtgggtggg gattgactcc tatgggtcgc 780
gaagcattac cacaagagct agtcggagcc tacttgtatt tggcatctga cgtgcacata 840
ttcacaatg gatgtgatat tcaagtagac ggtgggtaca cttgcgttta a 891

<210> 23
<211> 296
<212> PRT
<213> Kluyveromyces aestuarii

<400> 23
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20 25 30
Val Asn Tyr Leu Ser Leu Phe Ser Gln Lys Gly Lys Leu Thr Val Ile
35 40 45
Thr Gly Gly Ala Gly Ala Ile Gly Gly Ala Leu Cys Glu Gly Phe Ala
50 55 60
Ser Cys Gly Ser Asp Val Val Ile Leu Asp Tyr Lys Tyr Ser Pro Glu
65 70 75 80
Leu Ser Ser Val Leu Glu Ser Arg Tyr Gly Val Arg Ser Lys Ser Tyr
85 90 95
Gln Val Asp Ile Thr Ser Ser Glu Asp Val Lys Leu Val Val Ala Lys
100 105 110
Ile Leu Glu Asp Phe Pro Asp Arg Asp Ile Asn Thr Phe Val Ala Asn
115 120 125
Ala Gly Ile Ala Trp Thr Asn Gly Ser Ile Leu Asn Glu Asn Ala Thr
130 135 140
Pro Asp Val Trp Lys Arg Val Met Asp Val Asn Val Gln Gly Thr Tyr
145 150 155 160
His Cys Ala Lys Tyr Val Ala Glu Val Phe Lys Gln Gln Gly His Gly
165 170 175
Asn Leu Ile Leu Thr Ala Ser Met Ser Ser Tyr Ile Ser Asn Val Pro
180 185 190
Asn Tyr Gln Thr Cys Tyr Asn Ala Ser Lys Ala Ala Val Arg His Met

8

195	200	205
Ala Lys Gly Phe Ala Val Glu Phe Ala His Leu Thr Asn Pro Ala Gly		
210	215	220
Lys Ile Arg Cys Asn Ser Val Ser Pro Gly Tyr Thr Asp Thr Ala Leu		
225	230	235
Ser Ala Phe Val Pro Val Glu Gln Arg Ala Gln Trp Trp Gly Leu Thr		
245	250	255
Pro Met Gly Arg Glu Ala Leu Pro Gln Glu Leu Val Gly Ala Tyr Leu		
260	265	270
Tyr Leu Ala Ser Asp Ala Ala Ser Phe Thr Asn Gly Cys Asp Ile Gln		
275	280	285
Val Asp Gly Gly Tyr Thr Cys Val		
290	295	

<210> 24

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 24

aattctcgag taatctagag gaattctaaa a

31

<210> 25

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 25

ctagtttttag aattcctcta gattactcga g

31

<210> 26

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 26

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<210> 27

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificially Synthesized Primer Sequence

<400> 27

ggtaagcttt cattaaccgc ggcctgcctg

30

<210> 28
 <211> 786
 <212> DNA
 <213> *Bacillus subtilis*

<400> 28
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 aaggcgatgg ccatttcgctt cggcaaggag caggcaaaag tggttatcaa ctattatagt 120
 aataaacaag atccgaacga ggtaaaagaa gaggtcatca aggcgggcyg tgaagctgtt 180
 gtcgtccaag gagatgtcac gaaagaggaa gatgtaaaaa atatcgtgca aacggcaatt 240
 aaggagttcg gcacactcga tattatgatt aataatgccg gtcttgaaaa tcctgtgcca 300
 tctcacgaaa tgcgcgtcaa ggattgggat aaagtcatcg gcacgaactt aacgggtgcc 360
 tttttaggaa gccgtgaagc gattaaatat ttcgtagaaa acgatataca gggaaatgtc 420
 attaacatgt ccagtgtgca cgaagtgatt ccttgggcgt tatttgcca ctatgcggca 480
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 attcgcgtca ataatatgg gccaggtgcy atcaacacgc caatcaatgc tgaaaaattc 600
 gctgacccta aacagaaaagc tgatgtagaa agcatgattc caatgggata tatcgccgaa 660
 ccggaggaga tcgccgcagt agcagcctgg cttgcttcga aggaagccag ctacgtcaca 720
 ggcatacagt tattcgcgga cggcgggtatg acacaatatc cttcattcca ggcaggccgc 780
 ggtaa 786

<210> 29
 <211> 261
 <212> PRT
 <213> *Bacillus subtilis*

<400> 29
 Met Tyr Pro Asp Leu Lys Gly Lys Val Val Ala Ile Thr Gly Ala Ala
 1 5 10 15
 Ser Gly Leu Gly Lys Ala Met Ala Ile Arg Phe Gly Lys Glu Gln Ala
 20 25 30
 Lys Val Val Ile Asn Tyr Tyr Ser Asn Lys Gln Asp Pro Asn Glu Val
 35 40 45
 Lys Glu Glu Val Ile Lys Ala Gly Gly Glu Ala Val Val Val Gln Gly
 50 55 60
 Asp Val Thr Lys Glu Glu Asp Val Lys Asn Ile Val Gln Thr Ala Ile
 65 70 75 80
 Lys Glu Phe Gly Thr Leu Asp Ile Met Ile Asn Asn Ala Gly Leu Glu
 85 90 95
 Asn Pro Val Pro Ser His Glu Met Pro Leu Lys Asp Trp Asp Lys Val
 100 105 110
 Ile Gly Thr Asn Leu Thr Gly Ala Phe Leu Gly Ser Arg Glu Ala Ile
 115 120 125
 Lys Tyr Phe Val Glu Asn Asp Ile Lys Gly Asn Val Ile Asn Met Ser
 130 135 140
 Ser Val His Glu Val Ile Pro Trp Pro Leu Phe Val His Tyr Ala Ala
 145 150 155 160
 Ser Lys Gly Gly Ile Lys Leu Met Thr Glu Thr Leu Ala Leu Glu Tyr
 165 170 175
 Ala Pro Lys Gly Ile Arg Val Asn Asn Ile Gly Pro Gly Ala Ile Asn
 180 185 190
 Thr Pro Ile Asn Ala Glu Lys Phe Ala Asp Pro Lys Gln Lys Ala Asp
 195 200 205
 Val Glu Ser Met Ile Pro Met Gly Tyr Ile Gly Glu Pro Glu Glu Ile
 210 215 220
 Ala Ala Val Ala Ala Trp Leu Ala Ser Lys Glu Ala Ser Tyr Val Thr

225 230 235 240
Gly Ile Thr Leu Phe Ala Asp Gly Gly Met Thr Gln Tyr Pro Ser Phe
 245 250 255
Gln Ala Gly Arg Gly
 260